

CLAIMS

1. A variant of the PapM polypeptide of bacteria of the *Streptomyces* genus, which derives from the sequence of the wild-type PapM polypeptide of
5 *S. pristinaespiralis* or of a homologous polypeptide by replacement of one or more amino acids chosen from
 - a) the residue Gly 249 of the Pap M polypeptide of *S. pristinaespiralis*
 - b) the residue Thr 192 of the Pap M polypeptide of *S. pristinaespiralis*
 - c) a residue equivalent to (a) of (b) in a homologous peptide.
- 10 2. The variant as claimed in claim 1, which derives from the polypeptide sequence SEQ ID NO. 2 and which exhibits at least one substitution, at position 249, of a glycine with a serine.
3. The variant as claimed in claim 1, which derives from the polypeptide sequence SEQ ID NO. 2 and which exhibits at least one substitution, at position
15 192, of a threonine with an isoleucine.
4. The variant as claimed in claim 1, which derives from SEQ ID NO. 2 and which exhibits at least one substitution, at position 249, of a glycine with a serine and a substitution, at position 192, of a threonine with an isoleucine.
5. A nucleic acid encoding a variant as claimed in one of claims 1 to 4.
- 20 6. The nucleic acid as claimed in claim 5, which derives from the nucleotide sequence presented in SEQ ID NO. 1 or the sequences derived due to the degeneracy of the genetic code.
7. The nucleic acid as claimed in claim 6, which comprises at least one missense mutation upstream of the NPPY motif located at positions 193 to 196.
- 25 8. The nucleic acid as claimed in claim 7, wherein the missense mutation leads to a non-conservative amino acid change.
9. The nucleic acid as claimed in claim 8, which comprises at least one substitution of a cytosine at position 658 with a thymine (C658T).
10. The nucleic acid as claimed in claim 6, which comprises at least one
30 substitution of a guanine at position 828 with an adenine (G828A).

11. The nucleic acid as claimed in claim 8, which comprises at least one substitution of a guanine at position 828 with an adenine (G828A) and at least one substitution of a cytosine at position 658 with a thymine (C658T).
12. A recombinant DNA comprising a nucleic acid as claimed in one of
5 claims 5 to 11.
13. An expression vector which replicates autonomously and/or which integrates, which comprises a nucleic acid as claimed in one of claims 5 to 11 or a recombinant DNA as claimed in claim 12.
14. The vector as claimed in claim 13, which comprises all or part of the vector
10 pVRC1306 as represented in figure 11.
15. A host cell containing a nucleic acid as claimed in one of claims 5 to 11 and/or a recombinant DNA as claimed in claim 12 and/or an expression vector as claimed in either of claims 13 and 14.
16. The host cell as claimed in claim 15, which is chosen from *E. coli*,
15 *S. pristinaespiralis*, *Streptomyces olivaceus* ATCC12019, *Streptomyces ostreogriseus* ATCC27455, *Streptomyces mitakaensis* ATCC15297, *Streptomyces loïdensis* ATCC11415, *Streptomyces graminofaciens* and *Streptomyces diastaticus*.
17. A method for producing a variant as claimed in one of claims 1 to 4,
20 wherein a host cell as claimed in claim 15 or 16 is cultured and the polypeptide produced is recovered.
18. The use of a host cell as claimed in claim 15 or 16, expressing a variant as claimed in one of claims 1 to 4, in a bioconversion reaction.
19. The use of a nucleic acid as claimed in one of claims 5 to 11, for modifying
25 the proportion of the various B components of Streptogramins in a Streptogramin-producing strain.
20. The use as claimed in claim 19, for producing PIB, wherein the nucleic acid used is a nucleic acid encoding a variant as claimed in one of claims 1 to 4 and the Streptogramin-producing strain is a strain of *S. pristinaespiralis*.
21. The use as claimed in claim 19, for producing PIA, wherein the nucleic acid used is a nucleic acid encoding a variant as claimed in claim 8 and the Streptogramin-producing strain is a strain of *S. pristinaespiralis*.
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22. A method for producing one or more B components of Streptogramins, wherein:
- the papM gene is inactivated in a Streptogramin-producing or potentially Streptogramin-producing strain, and one or more copies of a nucleic acid encoding a papM variant as defined according to one of claims 1 to 4 are introduced,
 - said strain is cultured under conditions for producing Streptogramins, and
 - the B component(s) of the Streptogramins produced is (are) recovered.
23. The method as claimed in claim 22, wherein the Streptogramin-producing strain is a Pristinamycin-producing strain derived from *S. pristinaespiralis*, and preferably derived from the strain *S. pristinaespiralis* SP92 or from the strains *Streptomyces olivaceus* ATCC12019, *Streptomyces ostreogriseus* ATCC27455, *Streptomyces mitakaensis* ATCC15297, *Streptomyces loïdensis* ATCC11415, *Streptomyces graminofaciens* and *Streptomyces diastaticus*.
24. The method as claimed in either of claims 22 and 23, wherein the Streptogramin-producing strain is a strain which produces a small or undetectable amount of A components of Streptogramins.
25. The method as claimed in claim 23, wherein it involves a strain derived from *S. pristinaespiralis* SP213.
26. The method as claimed in one of claims 22 to 25, wherein the introduction of the nucleic acid encoding a PapM variant as claimed in one of claims 1 to 4 is carried out by replacement of the wild-type form of the papM gene.
27. The method as claimed in claim 26, which is of use for producing PIB, wherein the wild-type form of the papM gene is replaced with a nucleic acid encoding a variant as claimed in one of claims 1 to 4.
28. The method as claimed in one of claims 22 to 27, which is of use for producing PINH2, wherein the wild-type form of the papM gene is replaced with a nucleic acid encoding an inactive form of the papM polypeptide.
29. The method as claimed in claim 27, wherein the strain used comprises a papM gene exhibiting the double mutation (G828A) (C658T).
30. The method as claimed in claim 27, wherein the strain used comprises a

papM gene exhibiting the mutation (G828A).

31. The method as claimed in claim 27, wherein the strain used comprises a papM gene exhibiting the mutation (C658T).

5 32. A mutant strain of *S. pristinaespiralis*, which comprises a nucleic acid as claimed in one of claims 5 to 11.

33. The strain as claimed in claim 32, which is the strain strain [sic] *S. pristinaespiralis* SP217.

34. The strain as claimed in claim 32, which is the strain strain [sic] *S. pristinaespiralis* SP101.

10 35. The strain as claimed in claim 32, which is the strain *S. pristinaespiralis* SP218.

36. The use of the strain *S. pristinaespiralis* SP217, for producing PIB.

37. The use of the strain *S. pristinaespiralis* SP101, for producing PIB.

38. The use of the strain *S. pristinaespiralis* SP218, for producing PIB.

15 39. The use of the strain *S. pristinaespiralis* SP216, for producing PINH2.

40. A method for selecting a PapM polypeptide variant as claimed in one of claims 1 to 4, according to which:

- a chemical mutagenesis step is carried out on the papM gene of *S. pristinaespiralis* or a homologous gene cloned into a plasmid,
- 20 • a library is prepared by transforming a recipient strain with the plasmids on which mutagenesis was performed in step (a),
- the clones are selected which exhibit a methylation activity such that the ratio "r" of the amounts of methylation substrate converted per unit of time, with $r = \text{substrate 1} / (\text{substrate 1} + \text{substrate 2})$, exhibits a ratio of greater
- 25 than or equal to 0.6.